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<b>(21) International Application Number:</b> PCT/US98/05950 <b>(22) International Filing Date:</b> 26 March 1998 (26.03.98)  <b>(30) Priority Data:</b> 08/825,626                      31 March 1997 (31.03.97)                      US  <b>(71) Applicant:</b> OPTIMUM QUALITY GRAINS, L.L.C. [US/US]; 10700 Justin Drive, Des Moines, IA 50322 (US).  <b>(72) Inventor:</b> BERGQUIST, Richard, R.; 401 East 6th Street, El Paso, IL 61378 (US).  <b>(74) Agents:</b> FASSNACHT, Harold, J. et al.; Bullwinkel Partners, Ltd., Suite 1300, 19 South LaSalle Street, Chicago, IL 60603 (US).		<b>(81) Designated States:</b> BA, BG, CZ, RO, UA, YU, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> SYNTHETIC CORN HYBRID LP57.1  <b>(57) Abstract</b>  A synthetic hybrid corn plant having the designation LP57.1, produced by crossing two proprietary Pfister Hybrid Corn Company maize synthetics, LP44.1A-Ht/wx Reid and LP48.1B Lancaster. LP57.1 has the unique property of imparting high oil and high protein levels in the grain of certain normal and male sterile hybrids when used as a pollinator. LP57.1 is characterized by excellent cold tolerant seedling vigor for rapid emergence in cold soils, and excellent medium-late season adaptability to nick with medium-late maize hybrids that condition fast dry-down and superior grain quality in the recipient female grain parent. This invention thus relates to the synthetic hybrid plants and seeds of LP57.1, i.e., the synthetic hybrid produced by crossing the two aforementioned parental synthetics and the seed thereof, including advanced generation seed, variants, mutants, and modifications of LP57.1.		

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## SPECIFICATION

### Synthetic Corn Hybrid LP57.1

#### FIELD OF THE INVENTION

This invention is in the field of plant breeding. Specifically, this invention relates to a novel synthetic corn hybrid having the designation LP57.1 and useful in the proprietary TopCross™ grain production system described in pending United States patent applications serial nos. 07/615,839 and 08/464,249 by Bergquist et al.

#### BACKGROUND OF THE INVENTION

##### Uses Of Corn

Corn (Zea mays L.) is an important crop used as a human food source, animal feed, and as a raw material in industry. The food uses of corn, in addition to the human consumption of corn kernels, include products of both the dry milling and wet milling industries. The principal products of dry milling include grits, meal and flour. The principal products of wet milling include starch, syrups, and dextrose. A by product of both dry and wet milling is corn oil, which is recovered from corn germ. As animal feed, corn is used primarily as a feedstock for beef cattle, dairy cattle, swine, poultry, and fish.

Industrial uses of corn mainly consist of the use of corn starch produced by wet milling and corn flour produced by dry milling and the whole kernel fermentation for production of food-grade and industrial use ethanol. The industrial applications of

corn starch and flour are based on their functional properties, such as viscosity, film formation ability, adhesiveness, absorbent properties and ability to suspend particles. Corn starch and flour are used in the paper and textile industries, and as components in adhesives, building materials, foundry binders, laundry starches, sanitary diapers, seed treatments, explosives, and oil-well muds. Plant parts other than the corn kernels are also used in industry. For example, stalks and husks can be made into paper and wallboard, and corn cobs can be used for fuel and to make charcoal.

#### Principles of conventional plant breeding

Virtually all of the commercial corn produced in the United States is produced from hybrid seed. The production of hybrid seed first requires the development of elite corn inbred lines that possess good combining ability to produce agronomically superior hybrids. The majority of hybrid seed produced in the United States is of the single cross type, wherein two inbred lines are intermated, or crossed, to produce what is termed an  $F_1$  single cross hybrid. The resulting kernels from this intermating are then sold as seed to commercial growers who plant the seed and harvest the second generation, or  $F_2$  grain, for use on farm or for commercial sale.

The production of a conventional single cross hybrid seed involves controlling the direction of pollination from one inbred to the other to assure the production of predominantly hybrid (cross pollinated) seed. Typically directed pollination is accomplished by interplanting separate rows of female corn plants

with male corn plants. The female corn plants that are male sterile may be produced by genetic mechanisms which render the corn tassel nonfunctional or by detassling the plants in the field.

5           The development of corn hybrids requires the development of homozygous inbred lines or uniform synthetic populations of unique heterotic background, the crossing of these lines or synthetic populations, and evaluation of test crosses. Pedigree breeding and recurrent selection breeding programs are used to  
10 develop inbred lines and synthetic populations from breeding populations. Breeding various broad-based sources into breeding pools from which new inbred lines or synthetic populations are developed by inbreeding or random mating and selection of desired phenotypes. The new inbreds and/or synthetic lines are crossed  
15 with other inbred lines and/or synthetic populations and the hybrids from these crosses are evaluated to determine which have commercial value and agronomic usefulness.

          Pedigree breeding starts with the crossing of two genotypes, each of which may have one or more desirable characteristics that  
20 is lacking in the other or which complements the other. If the two original genotypes do not provide all of the desired characteristics, other sources can be included during the breeding. In the pedigree breeding method, superior plants are selfed or random mated and the resulting seed selected in  
25 successive generations. Pedigree records of ancestry are carefully maintained for each family and ear row selection

through succeeding generations. In the succeeding generations, the heterozygous condition of the corn germplasm gives way to homozygous true breeding lines as a result of inbreeding and selection. Typically in the pedigree method of breeding, five or more generations of inbreeding and selection is practiced:  $F_1$  to  $F_2$ ;  $F_2$  to  $F_3$ ;  $F_3$  to  $F_4$ ;  $F_4$  to  $F_5$ , etc.

Backcrossing can be used to improve an inbred line by transferring a specific desirable trait from one inbred or source to another inbred that lacks that trait. This can be accomplished, for example, by first crossing a superior inbred (recurrent parent) to a donor inbred (nonrecurrent parent). The donor inbred carries (donates) the appropriate gene(s) for the desired trait to the next generation. After five or more backcross generations with selection for the desired trait, the inbred will be heterozygous for loci controlling the characteristic being transferred, but will be like the superior parent for most or almost all other genes. The last backcross generation can be selfed to produce a pure breeding progeny for the gene(s) being transferred.

An important consequence of the homozygosity and homogeneity of the inbred lines is that the hybrid between any two inbreds will always be the same. Once the inbreds or synthetics that give the best hybrid have been identified, the hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred or synthetic parents is maintained.

A synthetic hybrid consists of an array of similar genotypes

that were identified from intercross tests and bulked into a random mating population having a desired phenotype. The intercrosses between two different heterotic groups results in the continuous production of a specific synthetic hybrid of desired phenotype.

As previously noted, a single cross hybrid is produced when two unrelated inbred or synthetic lines are crossed to produce the  $F_1$  progeny. A three-way cross hybrid is produced from three inbred lines (or synthetics) where two of the inbred lines (or synthetics) are crossed ( $A \times B$ ) and then the resulting  $F_1$  hybrid is crossed with the third inbred (or synthetics) ( $A \times B$ ) $\times$ C. A double cross hybrid is produced from four inbred lines (or synthetics) by crossing pairs ( $A \times B$ ) and ( $C \times D$ ) and then crossing the two  $F_1$  hybrids ( $A \times B$ ) $\times$ ( $C \times D$ ).

Much of the hybrid vigor exhibited by  $F_1$  hybrids is lost in the next generation ( $F_2$ ). Consequently, seed (grain) from hybrid varieties is not used for planting stock.

The objective of typical plant breeding is to combine in a single variety/hybrid the desirable traits of the parental lines. For field crops such as corn, these desirable traits may include resistance to diseases, insects, herbicide tolerance, and tolerance to heat and drought, reducing time to crop maturity, and improved agronomic quality. With mechanical harvesting of many crops, uniformity of plant characteristics such as germination time and stand establishment, growth rate, and fruit/seed size are also desirable.

The problem with conventional breeding techniques is that there are several grain quality traits, such as high oil content, that cannot readily be combined in a high-yielding single cross hybrid. The present invention, when used as a pollinator, imparts desirable grain quality characteristics, such as high oil content, to the resulting  $F_1$  grain without significant loss of yield. This heretofore was not possible because these desirable grain quality characteristics in hybrids usually have been associated with low yield and poor agronomic characteristics.

#### Synthetic varieties

Corn has male flowers, located on the tassel, and female flowers, located on the ear, of the same plant. Because of this monoecy, corn plants can be bred by both self-pollination and cross-pollination techniques. Corn is self-pollinated if pollen from one flower is transferred to the same or another flower on the same plant. Corn is cross-pollinated if the pollen comes from a flower on a different plant.

Plants that have been self-pollinated and selected for uniform type over many generations become homozygous at almost all gene loci and produce a uniform population of true breeding progeny. Cross pollination between two homozygous lines produces a uniform population of hybrid plants that nevertheless may be heterozygous for many gene loci. A cross between two plants that are each heterozygous for a number of gene loci will produce a population of hybrid plants that differ genetically and will not be uniform.



Natural pollination occurs when wind blows pollen from tassels to silks that protrude from tops of the incipient ears on plants of the same genotype and different genotype, resulting in both self- and cross- pollination. When a population of  
5 genotypes are combined from all possible intercrosses among a number of selected genotypes and are allowed to open pollinate, the result is called a synthetic variety. A synthetic variety is made up of genotypes which previously have been tested for their ability to produce a superior progeny when crossed in all  
10 combinations.

Corn plants may be maintained as an outcrossing synthetic population that is much less homogeneous than a self-pollinated group. Every plant in such a group is certain to be heterozygous at many or most loci, and this heterozygosity must either be  
15 maintained during a breeding program or restored at the end of the program, if productivity is to be satisfactory. The main requirement in maintaining a synthetic line is that a sufficient number of plants of heterozygous background be maintained to recover the gene frequencies that are desired for the synthetic  
20 population so as to prevent genetic drift toward undesired gene frequencies.

#### The desirability of high oil content grain

The concentration of oil in most varieties of corn ranges from less than 3.0 percent to 4.5 percent at 0% moisture.  
25 Embryos of ordinary corn can contain 30 percent oil, while embryos of high oil corn strains can contain as much as 50

percent oil and are much larger in size than ordinary corn embryos.

There are several reasons for wanting to develop a method for growing corn that is high in oil content. First, corn oil is a premium oil and regularly more valuable than starch, the other major component of corn kernels. Second, high oil corn possesses a higher available energy content than ordinary corn, and thus is a more valuable feed for poultry and livestock. In animal feeding trials it has been found that less high oil corn is required per unit of gain than is required with ordinary corn. In addition, high oil corn requires substantially less soybean meal to balance a typical animal diet, and may be used to replace oil containing additives in animal feed.

Additional impetus was given to breeding corn for high oil by the development of wide-line nuclear magnetic resonance spectroscopy (NMR) and near-infrared spectroscopy (NIR) as analytical tools for the nondestructive analysis of bulk or single kernel samples that can be carried out in as little as two seconds. The development of such tools made it much easier and much quicker to determine the oil content of grain, thereby encouraging experimentation in the area of breeding for high oil.

Thus there exists at present a growing market for corn having high oil, increased protein and other special end-use properties which is not met by corn of standard composition. The diverse types of corn available to plant breeders provides a potential for modification of quality and quantity of grain

protein, starch, and oil. Corn now can be developed to more precisely meet the specific nutritional requirements of animals or to meet particular industrial needs.

The TopCross™ grain production system

5           Unfortunately, high oil is a property that cannot readily be achieved in a high yielding single-cross hybrid. This is because oil content, while being a moderately heritable trait, is influenced by a series of oil genes that have additive effects on oil content and occur at a complex of loci in at least eight  
10 linkage groups that influence the amount of oil in the grain progeny. Obtaining a hybrid having all or most of these oil genes can take many years of breeding. Further increasing the difficulty of breeding for high oil content is the fact that the grain yield of higher oil hybrids is generally inferior when  
15 compared to elite dent corn hybrids.

          A method of producing a high yield of corn having high oil content without requiring years of breeding is described in Bergquist et al. United States Patent Application Serial No. 07/615,839. The primary aspect of this method, known as the  
20 TopCross™ grain production system, is the interplanting of a pollinator corn plant possessing the characteristics for significantly increasing oil and protein levels in the resulting grain with a male sterile hybrid corn plant. The resulting grain possesses an oil content much higher than would be expected for  
25 self- or cross- pollination of the fertile version of the hybrid corn plant.

In practice, the seed of the pollinator with improved grain quality traits is blended in small amounts with seed of an elite male sterile grain parent hybrid, but with sufficient pollinator seed to permit abundant pollen production for fertilization of the male sterile grain parent hybrid. The relatively low ratio of pollinator seed to male sterile grain parent seed (less than one pollinator plant to every three grain parent plants) takes advantage of the higher grain yield potential of the elite grain parent hybrid while assuring a sufficient population of pollinator plants to pollinate the male sterile grain parent plants.

#### Need for superior pollinators

Critical to the success of the TopCross™ grain production system is the use of a pollinator capable of enhancing the grain quality traits of the F<sub>1</sub> offspring. To obtain such pollinators, the corn breeder must select and develop corn plants that have the traits that result in superior inbred and synthetic parental lines.

The pollinator for the TopCross™ grain production system need not be genetically homozygous (inbred) or even uniform in appearance, and need not be selected for genetic combining ability with female plants. However, the pollinator should have uniform desirable grain quality characteristics, such as high oil, that will influence the grain quality characteristics of the F<sub>1</sub> offspring, and the ability to pollinate the female plants. A hybrid obtained by crossing two synthetic populations of

different heterotic backgrounds results in a synthetic hybrid with predictable heterozygosity and genetic variability among plants that is particularly useful as a male pollinator in blends with male sterile hybrid grain parents in the TopCross™ grain production system. Some genetic variability is desirable because it extends the flowering period of the pollinator. LP57.1 was developed to achieve these characteristics.

#### Advantages of synthetic hybrids

The use of synthetic hybrids (such as LP57.1) as TopCross™ grain production system pollinators affords a number of advantages over the use of hybrids produced from single crosses. For instance, synthetic hybrids can be developed more rapidly than commercial hybrids. Specifically, the use of a synthetic population can more rapidly establish stability of dominant oil genes, thus by-passing the many generations of inbreeding that is required to produce inbreds for making single cross hybrids.

Second, synthetic hybrids often have excellent vigor comparable to that of commercial hybrids. Inbreds, by contrast, typically lose vigor with each successive generation of inbreeding. This is an important advantage of synthetics because pollinator vigor is critical for ample pollen shed at the time of silking in the TopCross™ grain production system. Synthetic hybrid LP57.1 expresses cold vigor in seedling growth stages greater than even most open pollinated synthetic populations.

Third, a synthetic variety, utilizing heterosis in which pollination control is a factor, is more likely to disperse

pollen over a longer period of time than a single cross hybrid. The predictable greater variability of synthetic varieties as compared with single crosses permits more flexibility to meet the changing growing conditions typical of field production. In addition, because of the longer flowering period, fewer synthetic pollinators need be developed to be used in blends with many different grain parents.

Fourth, the synthetic hybrid pollinator is more easily produced during periods of heat and drought stress on dryland production than a single-cross hybrid using less vigorous inbred seed stocks. For example, in non-irrigated dryland field tests conducted during 1993 and 1994, production of synthetic hybrid seed remained relatively constant at about 55 bushels per acre despite the fact that rainfall accumulation during the critical months of May, June and July fell from 40.84 cm in 1993 to 13.82 cm in 1994. Over the same period, single cross seed production using inbred seed stocks fell to less than 25 bushels per acre in 1994 from 55 bushels per acre in 1993.

Fifth, the single cross synthetic hybrid pollinator which results from the cross of two parental synthetic populations, A x B, is more quickly produced in a single generation compared to a three-way cross pollinator (A x B)C that requires an additional plant generation to produce the hybrid three-way cross pollinator. For example, the A x B synthetic hybrid is simply produced in a single plant growing generation in the production of LP57.1 single-cross synthetic hybrid while the three-way cross

synthetic hybrid pollinator would require an additional plant generation to produce the final hybrid (A x B) crossed to the parental C-population to produce a synthetic three-way hybrid cross designated (A x B)C.

#### SUMMARY

According to the invention, there is provided a novel synthetic corn hybrid, designated LP57.1, that when used to pollinate an elite male sterile hybrid grain parent, produces commercial grain exhibiting improved quality grain traits, including high oil and protein. LP57.1, when used to pollinate male sterile hybrid grain parents that are harvested as whole plants at approximately 50% plant moisture, produces commercial fodder that expresses improved feeding quality traits, including improved efficiency and rate of weight gain. The invention thus relates to the seeds, plants and plant parts of LP57.1; to plants regenerated from tissue culture of the plants or plant parts of LP57.1; to a method of producing LP57.1 by crossing synthetic LP44.1A-Ht/wx-Reid and LP48.1B-Lancaster synthetics; and to a method of producing high oil grain using LP57.1 as a pollinator.

#### DEFINITIONS

In the description and examples that follow, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided:

Adaptation. The process by which individuals (or parts of individuals) or populations change in form or function to better survive under given environmental conditions. Also the result of this process.

5       Anthesis. The period or act of flowering.

Backcross. The cross of a hybrid to either one of its parents. The offspring of such a cross is referred to as the backcross generation.

10       Backcross Method of Breeding. A system of breeding carried out by several generations of backcrossing to one of the parents of a hybrid and subsequent selection. The characteristics of the recurrent parent are retained for the most part, and characteristics from the nonrecurrent parent are added.

15       Barren Plants. Plants that lack ears, typically measured in number of plants per plot.

Brittle Stalks. This is a measure of the stalk breakage near the time of pollination of the hybrids, and is an indication of whether a hybrid would snap or break at the time of flowering under severe winds.

20       Bulk Method of Breeding. The growing of segregating generations of a hybrid of self-pollinating crops in a bulk, with or without mass selection, followed by individual plant selection in  $F_6$  or later generations.

25       Cob Score. The cob score is a rating of how well the grain is shelled off the cob and how badly the cob is broken up going through the combine. A high score indicates that the grain



shells off of the cob well, and the cob does not break.

Cytoplasmic Inheritance. Transmission of hereditary characters through the cytoplasm as distinct from transmission by genes carried by chromosomes. Detected by differing contribution  
5 of male and female parents in reciprocal crosses.

Diallel Cross. The crossing in all possible combinations of a series of genotypes.

Donor parent. The parent from which one or a few genes are transferred to the recurrent parent in backcross breeding.

10 Ear Height. The ear height is a measure from the ground to the top developed ear node attachment and is measured in centimeters.

Early Stand Count. This is a measure of the stand establishment in the spring and represents the number of plants  
15 that emerge on a per-plot basis for the hybrid.

Elite. This term characterizes a plant or variety possessing favorable traits, such as, but not limited to, high yield, good grain quality and disease resistance.

20 Embryo. The rudimentary plant in a seed. The embryo arises from the zygote. In high oil corn breeding, increases in oil content are accompanied by increases in embryo size.

Endosperm. The nutritive tissue formed within the embryo sac in seed plants. It commonly arises following the fertilization of the two primary endosperm nuclei of the embryo  
25 sac by the two male sperms. In a diploid organism the endosperm is triploid.

Expressivity. The degree of manifestation of a genetic character.

F<sub>1</sub>. The first generation of a cross.

5 F<sub>2</sub>. The second filial generation obtained by self-fertilization or crossing inter se of F<sub>1</sub> individuals.

F<sub>3</sub>. Progeny obtained by self-fertilizing F<sub>2</sub> individual. Subsequent generations F<sub>4</sub>, F<sub>5</sub>, etc.

10 Field corn: Varieties or cultivars of corn grown extensively on large acreage within a broad but defined geographic area for the production of grain and/or forage.

15 GDD Shed. The GDD is the number of growing degree days (GDD) or heat units required for an inbred line or hybrid to reach anthesis or pollen shed from the time of planting. Growing degree days are calculated by the Barger Method, where the heat units for a 24-hour period are:

$$\text{GDD} = \frac{(\text{Max.} + \text{Min.})}{2} - 50$$

20 The highest maximum used is 86 degrees F. and the lowest minimum used is 50 degrees F. For each hybrid it takes a certain number of GDDs to reach various stages of plant development. GDDs are a way of measuring plant maturity.

General combining ability. The average or overall performance of a genetic strain in a series of crosses.

25 Genotype. The fundamental genetic constitution of an organism.

Germ. The embryo of the corn kernel. It contains most of the oil found in the kernel.

Grain. Comprises mature corn kernels produced by commercial growers for purposes other than growing or reproducing the species.

5 Grain parent. Male sterile, elite hybrid that comprises a large majority of the plants in a TopCross production field.

Grain Parent Seed. Corn seed used to produce grain parent plants.

10 Grain Quality. This is a 1 to 5 rating for the general quality of the shelled grain as it is harvested based on the color of the harvested grain, any mold on the grain, and any cracked grain. Low scores indicate good grain quality.

15 Grain Quality Trait. This is any attribute of grain that is of commercial value. Such traits relate to the intermediate or final use of grain and include but are limited to the quantity or quality of oil, protein, starch, pigmentation, and fiber found in corn grain. Such traits also encompass physical attributes of the grain itself, such as grain texture, size, or hardness, among others. Certain of these compositional or physical attributes of grain correlate with functional attributes as well which are of commercial importance, such as susceptibility to breakage and spoilage, among others.

20 Heterozygous. A genetic condition existing when different alleles reside at corresponding loci on homologous chromosomes.

25 High oil source. A population of corn plants containing high oil genes used for corn breeding.

Homozygous. A genetic condition existing when identical

alleles reside at corresponding loci on homologous chromosomes.

Hybrid. (1) The progeny of a cross fertilization between parents belonging to different genotypes. (2) The first generation offspring of a cross between two individuals differing in one or more genes.

Hybrid Vigor. The phenomenon in which the cross of two stocks produce hybrids that show increased vigor-heterosis compared to the parent stocks.

Inbred. A substantially homozygous individual, variety or line.

Inbred Line. (1) A line produced by continued inbreeding. In plant breeding a nearly homozygous line usually originating by continued self-fertilization, accompanied by selection. (2) A relatively homozygous line produced by inbreeding and selection.

Kernel. The corn caryopsis comprising a mature embryo and endosperm which are products of double fertilization.

Line. A group of individuals from a common ancestry. A more narrowly defined group than a variety.

Male Sterility. A condition in which pollen is absent or non-functional in flowering plants.

MN RM. This represents the Minnesota Relative Maturity Rating (MN RM) for the hybrid and is based on the harvest moisture of the grain relative to a standard set of checks of previously determined MN RM rating. Regression analysis is used to compute this rating.

Moisture. The moisture is the actual percentage moisture of

the grain at harvest.

Multiple Genes. Two or more independent pairs of genes which produce complementary or cumulative effects upon a single character of the phenotype.

5 Ovule: A structure consisting of female reproductive tissue surrounded by maternal tissue.

Pedigree. A record of the ancestry of an individual, family, or strain.

10 Pedigree Breeding. A system of breeding in which individual plants are selected in the segregating generations from a cross on the basis of their desirability and on the basis of a pedigree record.

15 Penetrance. (1) The proportion of organisms heterozygous for a particular dominant gene or homozygous for a recessive which shows the phenotype under a set of specified environmental conditions. (2) Complete penetrance is the situation in which a dominant gene always produces a phenotypic effect or a recessive gene in the homozygous state always produces a detectable effect. (3) The frequency with which a gene produces a recognizable  
20 effect in individuals which carry the gene.

Percent Oil. The oil concentration of a corn kernel expressed on a dry weight basis.

Percent Yield. The yield obtained for a hybrid in terms of percent of the mean for the experiments in which it was grown.

25 Phenotype. (1) Physical or external appearance of an organism as contrasted with its genetic constitution (=genotype).

(2) A group of organisms with similar physical or external makeup. (3) The observed character of an individual without reference to its genetic nature.

Plant Height. This is a measure of the height of the hybrid from the ground to the tip of the tassel and is measured in centimeters. The data is given in percentage of mean of the experiments in which the hybrid was grown.

Polar Nuclei. Found in the ovule to give rise to the embryo and endosperm of the mature corn kernel.

Pollen. A structure which contains the two haploid sperm nuclei which fuse with the egg nucleus and polar nuclei found in the ovule to give rise to the embryo and endosperm of the mature corn kernel.

Pollinators. Male fertile corn plants that are used to pollinate male sterile hybrid corn plants.

Pollinator Seed. Corn seed that, when sown, germinates to produce pollinator plants.

Population. In genetics, a community of individuals which share a common gene pool. In statistics, a hypothetical and infinitely large series of potential observations among which observations actually made constitute a sample.

Predicted R.M. Predicted relative maturity is based on the harvest moisture of the grain. The relative maturity rating (R.M.) is based on a known set of checks and utilizes standard linear regression analyses referred to as the Minnesota Relative Maturity Rating System.

Quantitative Character. A character in which variation is continuous so that classification into discrete categories is not possible. Also, a character determined by a series of independent genes which are cumulative in their effect.

5       Recurrent Parent. Used in backcrosses to refer to the parent to which the first cross and successive backcrossed plants are crossed.

10       Root Lodging. The percentage of plants that root lodge; i.e., those that lean from the vertical axis at an approximately 30 degree angle or greater would be counted as root lodged.

Seed. Mature corn kernels produced for the purpose of propagating the species.

15       Seed parent. A corn line that is pollinated by pollen from pollinator plants, with hybrid corn seed resulting from this pollination.

Seedling Vigor. This is the visual rating (1 to 5) of the amount of vegetative growth after emergence at the seedling stage (approximately five leaves). A higher score indicates better vigor.

20       Selection Index. The selection index gives a single measure of a hybrids' worth based on information for up to five traits. A corn breeder may utilize his or her own set of traits for the selection index. One of the traits that is almost always included is yield.

25       Self-fertilization. The fusion of the female egg cell of one individual with a male sperm cell of the same individual.

Sibs: Progeny of the same parents derived from different gametes. Half sibs, progeny with one parent in common.

Single Cross. A cross between two genotypes, usually two genetically different inbred lines or synthetic lines.

5 Specific Combining Ability. The performance of specific combinations of genetic strains in crosses in relation to the average performance of all combinations.

10 Stalk Lodging. This is the percentage of plants that do not stalk lodge, i.e., stalk breakage, as measured by either natural lodging or pushing the stalks and determining the percentage of plants that break off below the ear. This is a relative rating of a hybrid to other hybrids for standability.

15 Stay Green. Stay green is the measure of plant health near the time of black layer formation (physiological maturity). A low score on a scale of 1 to 5 indicates better late-season plant health.

Synthetic Hybrid. Any offspring of a cross between two genetically unlike synthetic individuals or unlike individuals.

20 Synthetic Population. A genetically heterogeneous collection of plants of known ancestry created by the intermating of any combination of inbreds, hybrids, varieties, populations, races or synthetics.

25 Synthetic Variety. A variety produced by crossing inter se a number of genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination.



Test Cross. A cross of a double or multiple heterozygote to the corresponding multiple recessive to test for homozygosity or linkage.

Test Weight. The measure of the weight of the grain in pounds for a given volume (eg. bushel), adjusted for percent moisture.

Topcross. (1) A cross of a hybrid or synthetic or inbred to a multiple heterozygote of opposite corresponding multiple loci to obtain traits observed in the pollen donor parent. (2) A cross between a selection line, clone, etc., and a common pollen parent which may be a variety, inbred line, single cross, etc. The common pollen parent is called the topcross or tester parent. (3) In corn, a topcross is commonly an inbred-variety cross, an outcross of selections, clones, lines, or inbreds, to a common pollen parent.

TC Blend™. A trademark of E. I. DuPont de Nemours and Company for a physical seed mixture of pollinator seed and male sterile grain parent seed meeting specific quality criteria.

TC Blend Grain Parent. A grain parent plant, hybrid, variety, line, synthetic or synthetic hybrid that is used in the TopCross grain production system.

TC Blend Pollinator. A pollinator plant, hybrid, variety, line, synthetic or synthetic hybrid that is used in the TopCross grain production system.

TC Blend Pollinator Seed. Corn seed that, when sown, germinates to produce TC Blend pollinator plants.

TC Blend Seed Corn. A mixture of TC Blend pollinator seed and grain parent seed that, when sown, germinates to produce TC Blend pollinator plants and grain parent plants used in the TopCross grain production system.

5        TopCross™ Grain. The grain which results from the planting of TC Blend™ seed and having improved nutrient composition and grain quality. TopCross™ is trademark of E I. DuPont de Nemours and Company, Inc. for grain produced by the TopCross™ grain production system.

10        TopCross™ Grain Production System. A method of commercial corn production whereby a low yielding male fertile corn pollinator is blended at 8 to 20 percent of the total seed count with an elite high yielding male sterile hybrid grain parent and allowed to pollinate the male sterile grain parent to produce  
15        TopCross™ grain having increased food and feed nutritional value, thus capitalizing on the high yield potential of the male sterile hybrid grain parent while contributing the grain quality traits from the fertile pollinator.

20        Variety. A subdivision of a species. A group of individuals within a species which are distinct in form or function from other similar arrays of individuals.

Yield (Bushels/Acre). The yield in bushels/acre is the actual yield of the grain at harvest adjusted to 15.5% moisture.

#### 25                    DETAILED DESCRIPTION OF THE INVENTION

LP57.1 is a yellow dent corn, high oil single cross

synthetic hybrid having superior agronomic characteristics and the ability to impart desirable grain quality traits to a first generation grain when used as a pollinator in the TopCross™ grain production system.

5           Synthetic hybrid LP57.1 is produced by planting Pfister Hybrid Corn Company proprietary synthetic populations LP44.1A-Ht/wx Reid and LP48.1B Lancaster, allowing one synthetic to pollinate the other, and harvesting the resulting seed. Either synthetic parental population may be used as female parent or the  
10           male parent. Preferably, synthetic LP48.1B should be the male of the cross and synthetic LP44.1A-Ht/wx should be the female of the cross because of the earlier flowering characteristics of the LP44.1A-Ht/wx seed parent in hybrid synthetic LP57.1 production, thus facilitating straight away planting of both parental  
15           populations. Production planting of the male and female synthetics can be made at the same time when LP44.1A-Ht/wx is the female and LP48.1B the male due to the fact that male pollen is shed at a slightly later time when the female silks of LP44.1A-Ht/wx are receptive to the pollen of LP48.1B.

20           LP44.1A-Ht/wx Reid and LP48.1B Lancaster may be produced by conducting a series of crosses, selfings and backcrosses beginning with the crossing of LH132 and B73 with ASKC28 and B73 with UHOC3 (for LP44.1A-Ht/wx Reid) and the crossing of NC286 with ASKC28 (for LP48.1B Lancaster).

25           When produced according to the method disclosed herein, both LP44.1A-Ht/wx Reid and LP48.1B Lancaster breed true, that is,

produce an LP57.1 synthetic hybrid that is both reproducible and usable as a high oil TopCross™ pollinator.

### CHARACTERISTICS

Synthetic corn hybrid LP57.1 most closely resembles maize synthetics ASKC28, ASKC20 and UHO in characteristics of plant type, ear type, kernel type and usage, but LP57.1 is considerably later in maturity and expresses higher grain test weight with normal grain and dent phenotypes when compared to ASKC28.

LP57.1 synthetic hybrid has the following characteristics:

Table 1

#### SYNTHETIC HYBRID LP57.1 DESCRIPTION INFORMATION

Type:	Dent/High Oil
Region Best Adapted:	Most Southern, Central and Eastern Regions of USA Cornbelt

A. Maturity:	Zone 3
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Synthetic Maize Hybrid:	LP57.1
Heat Units from Emergence to Shed:	1093.0 GDD
Heat Units from Emergence to Silk:	1072.5 GDD
Heat Units from 50% Silk to 25% Kernel Moisture:	1350.5 GDD
Heat Units from Emergence to 25% Kernel Moisture:	2472.5 GDD
No. Reps.:	8

Where Heat Units\* =  $[(\text{Max. Temp.} (<86 \text{ Degrees F}) + \text{Min. Temp.} (>50 \text{ Degrees F})) / 2] - 50$

\*If Max. Temp. is greater than 86 degrees Fahrenheit, then 86 is used and if Min. Temp. is less than 50, then 50 is used. Heat units accumulated daily and can not be less than 0.

B. Plant Characteristics:

Height (to tassel tip):	259 cm
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	Length of Top Ear Internode:	14 cm
	Number of Ears per Stalk:	Slight two-ear tendency
	Ear Height (to base of top ear):	117 cm
	Number of Tillers:	None
5	Cytoplasm Type:	Normal
	Brace Root Color:	Green
	Number of Brace Root Nodes:	1
	Number of Brace Roots:	16

## 10 C. Leaf:

	Color:	Dark green
	Stalk Color:	Green
	Angle from Stalk:	41 Degrees
15	Marginal Waves (number):	2
	Number of Leaves (mature plants):	15
	Sheath Pubescence:	Smooth, pubescence absent
	Longitudinal Creases:	Absent
	Length (Ear node leaf):	92 cm
20	Width (widest point, ear node leaf):	10 cm
	Coleoptile Sheath Color:	Purple

## 25 D. Tassel:

	Number Lateral Branches:	15
	Branch Angle from central spike:	56 degrees
	Length (from flag leaf):	48 cm
	Peduncle Length (flag leaf to basal branches):	11 cm
30	Anther Color:	Yellow
	Glume Color:	Green

## E. Ear (Husked Ear Data Except When Stated Otherwise):

35	Length:	23 cm
	Weight (dried to 15.5% grain moisture):	206 gm
	Mid-point Diameter:	5 cm
	Silk Color (at silking):	Pale green
40	Husk Extension (Harvest stage):	Short, 2.0 cm (ear tip occasionally exposed)
	Husk Leaf (number):	5
	Husk Leaf Length:	5 cm
	Number of Husks:	13
45	Taper of Ear:	Average taper
	Position at Dry Husk Stage:	Upright
	Kernel Rows:	14; Distinct, straight
	Husk Color (fresh):	Light green
	Husk Color (dry):	Buff
50	Shank Length:	15 cm long
	Shank (No. of internodes):	11

Drying Time (unhusked ear):	Average
Husk Length:	26 cm
Husk Width:	18 cm

5 F. Kernel (dried, size from ear mid-point):

Length:	12 mm
Width:	7 mm
Thickness:	4 mm
Shape Grade (% rounds):	41% ( $\pm 3\%$ ) based on parent test
Pericarp Color:	Colorless
Aleurone Color:	Homozygous; yellow
Cap Color:	Yellow
Endosperm Color:	Yellow
Endosperm Starch Type:	Normal starch
Gm Wt/100 Seeds (unsized):	24 gm
Test Weight:	56 lbs./bu.
Percent Oil:	16.3 percent (1995)
	14.7 percent (1996)
Percent Protein:	14.2 percent (1995)
	13.1 percent (1996)
Percent Starch:	54 percent (1995)
	57 percent (1996)

25 G. Cob (dried, size from ear mid-point):

Diameter at mid-point:	2.7 mm (varied from 2.5 to 3.1 mm)
Strength:	Strong
Color:	Red, segregating for white and heterozygous for red.

35 H. Diseases:

Northern Leaf Blight:	Resistant, heterozygous for HT1 gene
Goss's Bacterial Wilt:	Intermediate
Southern Corn Leaf Blight:	Resistant
Heat Smut:	Susceptible
Common Smut:	Resistant
Stewart's Bacterial Wilt:	Intermediate
Corn Lethal Necrosis:	Susceptible
Northern Leaf Spot:	Intermediate
Common Northern Rust:	Intermediate
Southern Rust:	Susceptible
Eye Spot:	Intermediate
Gray Leaf Spot:	Tolerant
Fusarium Ear Rot:	Resistant
Fusarium Stalk Rot:	Intermediate

Diplodia Ear Rot:	Intermediate
Diplodia Stalk Rot:	Intermediate
MDMV:	Susceptible
Stunt:	Susceptible
Stay Green:	Intermediate

## I. Insects:

European Corn Borer:	Susceptible
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## J. Variety most closely resembling:

<u>Character</u>	<u>Synthetic and/or Hybrid,</u> <u>Inbred</u>
Maturity	B73, Pfister Hybrid 2650
Plant Type	ASKC28, UHO, ASKC20
Ear Type	ASKC28, UHO, ASKC20
Kernel Type	UHO, ASKC20, ASKC28
Usage	ASKC28, UHO, ASKC20

LP57.1 is adapted over a wide area of the Southern, Central and Eastern corn belt and can be used advantageously in seed blends with male sterile hybrids from approximately 108-118 relative maturity based on the Minnesota Relative Maturity Rating System for harvest moisture of the grain. LP57.1 cold test vigor was excellent in 1995 laboratory tests, exhibiting 93% emergence compared to 90% emergence for ASKC20, 92% emergence for UHOC3, and 83% emergence for ASKC28. Kernel size-out is also very good for LP57.1, with approximately 80 percent of the kernels falling in the medium flat category from seed produced on LP43.2B seed parent.

Although LP57.1's primary use would be as a pollinator in the TopCross™ grain production system with blends of medium to late maturing corn hybrid male sterile grain parents, it is also an acceptable male to be crossed to later or earlier maturing full season high oil synthetic populations to develop later or

earlier maturity pollinators for expanding the use of their genetics to fuller season maturity grain parents.

Pollen production is good with LP57.1. Under extreme heat and drought stress, LP57.1 did not top fire and has expressed excellent tolerance to tassel blasting (necrosis of top leaves and tassel, respectively). It sheds pollen for approximately eleven days and should be planted in 8 to 20 percent blends to ensure adequate pollen in commercial production of TopCross™ grain where it is used as a male pollinator.

LP57.1 has shown uniformity and stability within the limits of environmental influence for all traits as described in Table 1. LP57.1 has expressed segregation for red and white cob color because of the genetic differences of LP44.1A-Ht/wx Reid and LP48.1B Lancaster synthetic parent populations. LP57.1 is a synthetic hybrid that has been maintained by hand and cross pollination in isolated fields with continued observation of high oil for uniformity of dominant high oil genetics. Although segregating for cob color, glume color and plant height in test crosses, LP57.1 synthetic has consistently expressed high oil across different environments regardless of morphological phenotype.

LP57.1 is a medium-late flowering synthetic hybrid, broadly adapted to the corn growing areas of the Southern, Central and Eastern United States and Southern Europe. LP57.1 has high oil and excellent cold soil seedling vigor that conditions moderate rate of grain moisture drydown in the grain of the male sterile



hybrid grain parent.

#### BENEFITS OF LP57.1 AS A POLLINATOR

5 In field tests of the TopCross™ grain production system  
using LP57.1 as the pollinator and an elite male sterile hybrid  
grain parent, plants of both varieties were allowed to grow  
unmolested to maturity. Both varieties were allowed to continue  
to grow and natural cross-pollination occurred by the action of  
wind as is normal in most grasses, including corn (i.e.,  
10 excluding wheat). Of course, only pollen from the male parent  
synthetic hybrid, LP57.1, was available for pollination of the  
male sterile hybrid grain parent; the tassels, or pollen bearing  
flowering parts, of the grain parent having been rendered sterile  
by genetic or cytoplasmic mechanisms.

15 The fields where high oil TopCross™ grain was produced were  
well isolated from other corn fields to prevent any accidental  
contamination with ambient pollen. Such isolation techniques may  
be accomplished by timed delay with other hybrid corn production  
fields or by using a space distance pattern of more than 70 m  
20 from normal corn, well known to those skilled in the art of the  
seed corn industry.

Both varieties comprising the corn seed blend were allowed  
to continue to grow and be harvested. The ears harvested from  
the male sterile grain parent expressed the higher grain yield  
25 potential of the elite male sterile grain parent and the high  
oil, protein and grain density qualities of the pollen parent.

The grain from the male parent variety ears can also be harvested along with the grain of male sterile grain parent for high oil corn use.

Because the same oil source (i.e. ASKC28 and related UHOC3) was used in the development of the LP44.1A-Ht/wx Reid and LP48.1B Lancaster populations, only modest heterotic effects for yield were expressed in LP57.1. The low grain yields expected from synthetic hybrid LP57.1 pollinator dictated the need for a low percent of pollinator in the pollinator-grain parent seed blend (i.e., about 8 to 20 percent) so as to recover the grain quality traits of the pollinator, but a high enough percent to produce sufficient pollen to maintain the higher yield potential of the elite male sterile grain parent hybrid.

The field tests indicated that LP57.1 induces superior grain quality characteristics in the TopCross™ grain of the male sterile hybrid grain parent as a result of being pollinated by LP57.1. That is to say, the grain quality traits and high oil characteristics of LP57.1 were transferred to the grain of the male sterile grain parent.

#### EXAMPLES

In the examples and tables that follow, the characteristics of TopCross™ grain (grain yield, moisture percent, oil content, protein content and test weight) produced using LP57.1 as a pollinator are provided.

First year (1995) strip test trials were conducted at El

Paso, Illinois; Louisville, Illinois; Alburnette, Iowa; Mt.  
Vernon, Indiana and Oran, Missouri, comparing the characteristics  
of grain from eleven sterile hybrid grain parents pollinated by  
LP57.1 with the characteristics of grain produced from grow outs  
5 of the same hybrids in their fertile state. These results are  
presented in Table 2.

**TABLE 2**  
**FIRST YEAR (1995) TOPCROSS STRIP TEST TRIALS FOR LP57.1**

Hybrid Grain Parent	Grain Yield-Bu/A.				Moisture Percent				Oil Percent				Protein Percent				Test Weight (Lbs./Bu.)			
	Top- Cross	% of GP	Hybrid Self	Top- Cross	% of GP	Hybrid Self	Top- Cross	% of GP	Hybrid Self	Top- Cross	% of GP	Hybrid Self	Top- Cross	% of GP	Hybrid Self	Top- Cross	% of GP	Hybrid Self	Top- Cross	
Pfister Hybrid X577-Sdms																				
Mean*	101.6	81	122.9	20.3	106	19.4	7.61	169	4.49	10.2	108	9.4	54.4	95	57.2					
Pfister Hybrid X642-Sdms																				
Mean*	122.8	99	124.0	18.3	96	19.0	7.29	169	4.31	9.5	103	9.2	54.5	97	57.1					
Pfister Hybrid X570-Sdms																				
Mean*	112.6	88	127.0	21.1	108	19.9	6.12	144	4.25	9.9	105	9.4	55.0	96	57.1					
Pfister Hybrid 2640-Sdms																				
Mean*	110.7	84	132.2	16.2	92	17.3	7.27	151	4.80	9.2	103	8.9	54.0	97	56.0					
Pfister Hybrid 2650-Sdms																				
Mean*	129.6	91	143.0	16.9	94	17.8	6.71	142	4.71	8.6	97	8.9	54.0	96	56.0					
Pfister Hybrid X632-Sdms																				
Mean**	122.3	90	135.3	18.1	98	18.1	7.81	160	4.88	9.7	105	9.2	53.3	94	56.6					
Pfister Hybrid 2417-Sdms																				
Mean***	123.2	99	130.6	19.4	105	18.3	7.27	161	4.53	9.3	103	9.0	54.0	96	56.1					
Pfister Hybrid 3434-Sdms																				
Mean**	100.7	86	119.0	21.3	108	19.5	6.76	156	4.33	10.1	108	9.3	53.6	96	55.9					
Pfister Hybrid 3225-Sdms																				
Mean*	111.4	91	122.9	17.1	96	17.6	6.77	159	4.26	9.4	106	8.8	55.4	98	56.8					
Pfister Hybrid X575-Sdms																				
Mean*	124.1	104	119.5	17.8	101	17.4	7.46	158	4.73	9.3	104	8.9	52.7	94	56.1					

Table 2 (cont.)

Hybrid Grain Parent	Grain Yield-Bu/A.			Moisture Percent			Oil Percent			Protein Percent			Test Weight (Lbs./Bu.)		
	% of			% of			% of			% of			% of		
	Top- Cross	Hybrid Self	GP	Top- Cross	Hybrid Self	GP	Top- Cross	Hybrid Self	GP	Top- Cross	Hybrid Self	GP	Top- Cross	Hybrid Self	GP
Pfister Hybrid X595															
Mean*	120.3	106	117.0	17.4	95	18.3	7.48	158	4.73	9.4	107	8.8	54.9	98	56.0
Grand Mean	116.3	93	126.7	18.5	100	18.4	7.14	157	4.55	9.5	104	9.1	54.2	96	56.4

\* Three locations: El Paso, IL; Alburnette, IA and Newburgh, IN

\*\* Two locations: El Paso, IL and Newburgh, IN

\*\*\* Five locations: El Paso, IL; Louisvile, IL; Alburnette, IA; Mt. Vernon, IN and Oran, MO

Twelve second year (1996) strip test trials were conducted in the following locations: Avon, El Paso, Leroy and Oakford, Illinois; Newburgh, Quincy, Palmyer, Wadeville and Worthington, Indiana; Audubon, Charter Oak, Gilman and Grinnell, Iowa; Atchison, Manhattan and Meridian, Kansas; Howells and Meadow Grove, Nebraska; and Columbus, Ohio. The strip tests compared the characteristics of grain from eighteen Pfister hybrids rendered male sterile and pollinated by LP57.1 with the characteristics of grain produced from grow outs of the same Pfister hybrids in their fertile state. The hybrids used were Pfister hybrids 2680, 3333, X577, 3001, 2640, 3034, 2417, 3225, 2376, 2725, 2652, X574, X570, X642, X632 and X592. These results are presented in Table 3.

**TABLE 3**  
**SECOND YEAR (1996) TOPCROSS STRIP TEST TRIALS FOR LP57.1**  
 (multiple locations)

Hybrid Grain Parent	Grain Yield-Bu/A.			Moisture Percent			Oil Percent			Protein Percent			Test Weight (Lbs./Bu.)		
	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP
<b>Pfister Hybrid 2680-Sdms</b>															
Mean	156.8	98	161.6	20.9	99	21.0	6.9	156	4.5	7.7	101	7.6	53.1	99	53.7
<b>Pfister Hybrid 3333-Sdms</b>															
Mean	125.3	94	136.6	23.5	108	21.7	6.9	167	4.2	8.1	97	8.3	52.7	100	52.9
<b>Pfister Hybrid X577-Sdms</b>															
Mean	121.4	82	136.3	20.6	94	17.6	7.5	166	4.5	8.1	106	7.7	52.9	99	53.5
<b>Pfister Hybrid 3001-Sdms</b>															
Mean	146.6	94	158.0	21.4	102	20.8	7.2	162	4.5	7.9	103	7.7	53.0	99	53.6
<b>Pfister Hybrid 2640-Sdms</b>															
Mean	122.3	83	149.2	18.8	94	19.9	7.0	164	4.3	8.3	108	7.7	52.1	97	53.4
<b>Pfister Hybrid 3034-Sdms</b>															
Mean	128.6	95	137.4	20.9	101	20.7	7.6	183	4.2	7.7	102	7.6	51.7	98	52.8
<b>Pfister Hybrid 2417-Sdms</b>															
Mean	108.8	100	109.3	23.1	102	22.9	6.8	157	4.3	8.6	105	8.2	51.4	99	52.1
<b>Pfister Hybrid 3225-Sdms</b>															
Mean	111.7	88	127.6	21.3	96	22.1	7.0	161	4.4	8.4	108	7.9	53.3	98	54.3
<b>Pfister Hybrid 2376-Sdms</b>															
Mean	138.9	91	153.6	21.0	103	20.3	7.0	163	4.4	7.9	103	7.7	53.4	99	53.5
<b>Pfister Hybrid 2725-Sdms</b>															

Table 3 (cont.)

Hybrid Grain Parent	Grain Yield-Bu/A.			Moisture Percent			Oil Percent			Protein Percent			Test Weight (Lbs./Bu.)		
	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP	Top- Cross	Hybrid Self	% of GP
Mean	142.5	156.0	91	22.2	20.8	106	7.0	4.5	158	7.9	7.7	103	52.8	99	53.2
Pfister Hybrid 2652-Sdms															
Mean	152.2	164.8	93	20.9	21.0	102	7.5	4.2	181	8.0	7.7	104	51.6	99	52.1
Pfister Hybrid X574-Sdms															
El Paso, IL	135.6	140.5	96	25.6	23.2	110	6.6	4.1	161	7.7	7.8	99	48.6	95	50.9
Pfister Hybrid X570-Sdms															
Mean	141.2	155.8	91	25.0	23.1	108	6.6	4.0	164	8.3	8.3	101	51.6	100	51.5
Pfister Hybrid X642-Sdms															
El Paso, IL	130.5	141.1	92	23.9	22.3	107	7.0	4.2	167	8.2	8.4	98	50.6	95	53.0
Pfister Hybrid X632-Sdms															
Mean	148.3	133.3	111	21.7	21.9	100	7.6	4.3	179	8.0	7.7	104	52.1	100	52.2
Pfister Hybrid X592-Sdms															
Mean	124.2	133.0	96	18.5	19.7	94	7.2	4.2	172	7.6	8.3	93	53.6	97	55.2
Pfister Hybrid 2650-Sdms															
Mean	152.4	158.4	96	20.9	21.3	98	6.7	4.5	150	7.6	7.6	100	53.2	99	53.5
Pfister Hybrid 3975-Sdms															
Mean	123.9	149.8	84	24.3	21.9	111	6.6	4.4	152	8.4	7.7	109	51.4	97	52.7
Grand Mean	134.5	145.0	94	21.9	21.2	103	7.0	4.3	165	8.0	7.9	102	52.2	98	53.0



Data obtained from the first and second year strip tests included the following:

"Grain yield", expressed in bushels per acre for both the grain produced by the pollination of the male sterile hybrid by LP57.1 and for the fertile hybrid. Grain yield from the male sterile/LP57.1 seed blend is also expressed as a percent of the yield from the fertile grain parent yield (% of GP).

"Moisture Percent" (for grain produced from the seed blend and from the fertile grain parent), expressed as a percentage of total kernel weight. Relative grain moisture was determined by distillation on a Brown-Duvel moisture tester manufactured by the Seed Trade Reporting Bureau, Chicago, Illinois. Electronic moisture testers were calibrated against the moisture determinations of the Brown-Duvel moisture tester in field harvest tests.

"Oil Percent", the content of oil in the grain at harvest, expressed as a percent of total kernel weight. Oil percent was determined by NIR on a dry matter basis (0% moisture).

"Protein Percent", expressed as a percentage of protein in the grain on a dry matter basis as determined by NIR.

"Test Weight", expressed as the weight of the grain in pounds for a given volume (bushel) adjusted for percent moisture.

#### Yield Comparison

In the first year (1995) strip test trials (Table 2), blends of 8-9 percent pollinator seed and 91-92 percent male sterile

hybrid seed were planted and grown to maturity by expert professional research affiliate cooperators. Each seed blend was grown in 91 m long strips of a minimum of 48 rows in filed isolation and were sown in strips of 12, 24, 36, etc. rows for each hybrid blend, depending on the number of different male sterile hybrid grain parents that were compared with a common pollinator (i.e., LP57.1). In addition, fertile check hybrids were grown in the same field but at a distance of at least 70 m from the seed blends. At harvest, grain was harvested from a measured area and weighed. The moisture percentage of the grain was determined to compute yield and the bushels per acre number was adjusted to 15.5 percent moisture.

The primary grain parent hybrids used in the strip test trials were of the same maturity as synthetic hybrid pollinator LP57.1, while additional grain parents were included in related trials to illustrate modifications that may be practiced within the scope of this invention. All grain parent hybrids used in these examples were produced and marketed by and are readily available from Pfister Hybrid Corn Company of El Paso, Illinois.

The seed blends used consisted of about eight percent fertile LP57.1 synthetic hybrid pollinator seed that was dyed green and about ninety-two percent cytoplasmic male sterile grain parent hybrid seed that was dyed red. The dying facilitates monitoring of the seed blending.

At blends of 8-9 percent pollinator and 91-92 percent male sterile grain parent, little loss in total mean grain yield was

observed in first year trials compared to the total grain yield of the self pollinated grain parent. For example, the mean yield of grain produced by the pollination of male sterile Pfister Hybrid 2417-Sdms by LP57.1 was 123.2 Bu/A, only slightly less than the mean yield of grain from the fertile X642 hybrid alone (130.6 Bu/A). The overall mean yield of grain produced by the pollination of all eleven hybrids by LP57.1 was 116.3 Bu./A., or 92% of the overall mean yield of the eleven fertile hybrids alone (126.7 Bu/A.).

In second year (1996) trials (Table 3), the grain yield from blends of 8-9 percent pollinator and 91-92 percent male sterile grain parent averaged 94% of the yield from the fertile grain parent alone (134.5 Bu./A. compared to 145.0 Bu./A.).

#### Moisture Of TopCross™ Grain

Conventional high oil hybrids traditionally express higher grain moisture at harvest and are slower to dry down than lower-oil dent hybrids of the same maturity. To test this concept of higher moisture associated with higher oil content of grain, comparisons were made of moisture at harvest of grain resulting from the pollination by LP57.1 of male sterile Pfister hybrids and grain resulting from the self pollination of the comparable fertile Pfister hybrids.

In first year trials (Table 2), the mean grain moisture percent at harvest from eleven sterile grain parent hybrids pollinated by LP57.1 (18.5%) was only slightly higher than the

mean grain moisture percent from the fertile grain parent hybrids alone (18.4%), providing only weak support for the conventional theory regarding the relationship between oil content and grain moisture.

5        In second year trials (Table 3), mean grain moisture percent at harvest from the sterile grain parent hybrids pollinated by LP57.1 was slightly higher, at 21.9%, than the mean grain moisture percent from the fertile grain parent hybrids alone (21.2%).

#### Oil Content Of TopCross™ Grain

Comparisons of the oil content of TopCross™ grain were made against the oil content of grain from corresponding fertile hybrids in first and second year strip tests.

10        The oil contents of grain produced in the first year (1995) strip tests of eleven Pfister male sterile hybrids pollinated by LP57.1 are presented in Table 2. Comparisons of the overall mean oil content of the TopCross grain to the overall mean oil content of the grain from the fertile checks of the corresponding hybrids in tests revealed about a 2.6% absolute increase in oil seen in  
15        the TopCross grain (7.14% compared to 4.55% for the eleven self pollinated Pfister hybrids).

20        In second year (1996) comparisons (Table 3), the mean oil content of grain produced by eighteen male sterile Pfister  
25        hybrids pollinated by LP57.1 was 163 percent higher than the mean oil content of grain produced from the self pollination of the

fertile hybrids alone (7.0% compared to 4.3%).

### Protein Content

In the first and second year strip tests, protein content of the TopCross™ grain (i.e., the grain resulting from the pollination of the male sterile hybrid by LP57.1) was compared to the protein content of grain produced from open pollinated fertile hybrid checks. Comparison of the mean protein contents indicated that LP57.1 increased protein in the TopCross™ grain compared to the grain from the fertile grain parent check.

For example, in first year strip testing (Table 2), the overall mean protein level of the grain produced by the pollination of eleven Pfister hybrids by LP57.1 (9.5%) was 104 percent of the overall mean protein level of the grain produced by the self pollination of the hybrids (9.1%). In second year strip tests (Table 3), the overall mean protein level from grain produced by the pollination of eighteen Pfister hybrids by LP57.1 (8.0%) was 101 percent of the mean protein level in grain produced by the self pollination of the fertile hybrids (7.9%).

### Test Weight

Test weight of grain is a function of kernel density. In first year strip tests, comparisons of the test weight of TopCross™ grain resulting from the pollination by LP57.1 of male sterile Pfister hybrids was made against the test weight of grain resulting from the self pollination of the comparable fertile

hybrids. As shown in Table 2, the overall mean test weight of TopCross grain across five locations was 54.2 lbs./Bu., or 96% of the mean test weight of the selfed hybrids (56.4 lbs./bu.). In second year strip tests, the overall mean test weight of TopCross grain produced by the pollination of eighteen Pfister hybrids by LP57.1 was 52.2 lbs./Bu., or 98% of the overall mean test weight of the selfed hybrids.

LP57.1 conditions a slight loss in test weight in the TopCross grain which is reflected in a slight yield penalty. This is because LP57.1 was developed by crossing LP44.1A-Ht/wx Reid, a soft starch grain phenotype, with LP48.1B-Lancaster, a normal starch grain phenotype. This cross yields a heterogeneous condition in the LP57.1 pollinator, i.e., the presence of types of male pollen gametes - soft starch and normal starch. When LP57.1 is used as a TopCross pollinator, the two types of male pollen gametes fertilize the male sterile hybrid grain parent. As a result, some of the TopCross grain expresses lower test weight (female ovules fertilized by male gametes with soft starch genotype) and the remainder of the grain expresses normal test weight (female ovules fertilized by male gametes with normal starch genotype). Overall, the grain exhibits a mid-parent mean that results in a slight loss in test weight which is reflected in a slight yield penalty.

#### Tassel-Silk Synchronization

The success of the TopCross™ grain production system relies

in large part on the synchronization of pollen shed from the pollinator with the extrusion of silks from the male sterile grain parent hybrid, which is termed nicking.

Table 4 shows the pollen shedding dates and the silk  
5 extrusion dates of LP57.1. The data reveals that LP57.1 shed pollen over an eleven day period, from July 22 through August 1, and extruded silks over the same period. The 50 percent pollen shedding date (i.e. the date by which 50% or more of the plants shed pollen) for LP57.1 was July 27, resulting from an  
10 accumulation of 1274.5 GDD. The 50 percent silk extrusion date was July 25, resulting from an accumulation of 1254.0 GDD. In the same experiment Pfister Hybrid 3001 expressed 50% silk extrusion on July 25, which resulted from an accumulation of 1245.5 GDD.

15

**TABLE 4**

**Frequency Distribution of Pollen Shedding Period  
and Frequency Distribution of Silk Extrusion Period for LP57.1 (1996)**

Date and Percent of Population							
	July 22	July 23	July 24	July 25	July 26	July 27	July 28
Percent of LP57.1 Plants Shedding Pollen	2	1	6	15	19	17	17
Cumulative	2	3	9	24	43	60	77
Percent of LP57.1 Plants Extruding Silks	1	9	2	9	15	24	13
Cumulative	1	10	12	21	36	60	73
	July 29	July 30	July 31	Aug 1	Dead	Plants Observed	
Percent of LP57.1 Plants Shedding Pollen	6	10	3	3	0.5	185	
Cumulative	83	93	96	99	100		
Percent of LP57.1 Plants Extruding Silks	7	12	5	3	0	186	
Cumulative	80	92	97	100	100		



Deposit Information

Applicant has made available to the public without restriction a deposit of at least 2500 seeds of synthetic hybrid LP57.1 with the American Type Culture Collection (ATCC),  
5 Rockville, MD. 20852, ATCC Deposit No. 97874. Synthetic parental seed stocks of LP44.1A-Ht/wx Reid and LP48.1B Lancaster have also been made available to the public without restriction from a deposit of at least 2500 seeds of each synthetic population with the American Type Culture Collection (ATCC) under Deposit No.  
10 97888 for LP44.1A-Ht/wx and No. 97887 for LP48.1B.

The seeds deposited with the ATCC are taken from the same deposits maintained by Pfister Hybrid Corn Company, Box 187, 187 North Fayette Street, El Paso, Illinois 61738, since prior to the filing date of this application. The deposits will be maintained  
15 in the ATCC depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the effective life of the patent, whichever is longer, and will be replaced if a deposit becomes nonviable during that period.

20 ASKC28 and UHOC3 have been previously deposited with the ATCC. LH132, NC286, UHOC3 and B73 are publicly available maize materials.

25 Although the foregoing invention has been described in some detail by way of illustration and examples for purposes of clarity and understanding, it will be obvious that certain

modifications and alternative embodiments of the invention are contemplated which do not depart from the spirit and scope of the invention as defined by the foregoing teachings and appended claims.

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>45</u> , lines <u>1-22</u>	
<b>B. IDENTIFICATION OF DEPOSIT</b> <span style="float: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></span>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 12301 Parklawn Drive Rockville, Maryland 20852 United States of America	
Date of deposit 10 February 1997	Accession Number ATCC #97874
<b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <span style="float: right;">This information is continued on an additional sheet <input type="checkbox"/></span>	
In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)	
<b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (If the indications are not for all designated States)	
<b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications, e.g., "Accession Number of Deposit")	

<b>For receiving Office use only</b>	
<input checked="" type="checkbox"/> This sheet was received with the international application	
Authorized officer	<i>Misty Walker</i> International Division 703-305-3682 misty.walker@uspto.gov

<b>For International Bureau use only</b>	
<input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer	

What is claimed is:

1. A novel synthetic hybrid corn seed designated LP57.1 and having ATCC accession No. 97874, or any corn line derived therefrom.

5 2. A novel synthetic hybrid corn plant and its parts produced by the seed of claim 1.

3. Corn plants regenerated from tissue culture of the synthetic hybrid corn plant of claim 2.

4. Pollen of the synthetic hybrid corn plant of claim 2.

10 5. Tissue culture according to claim 3 comprising regenerable cells of a plant part selected from the group consisting of meristematic tissues, anthers, leaves, embryos, protoplasts, and pollen.

15 6. A corn plant regenerated from regenerable cells of a tissue culture according to claim 5.

7. A synthetic hybrid corn plant having all the phenotypic, genotypic and physiological characteristics of the seed of claim 1.

20 8. A novel method for producing a synthetic hybrid corn seed having the designation LP57.1 comprising the steps of:

a) planting in pollinating proximity seeds of corn synthetic lines LP44.1A-Ht/wx Reid and LP48.1B Lancaster;

b) cultivating corn plants resulting from the planting until the time of flowering;

25 c) emasculating the flowers of the plants of either synthetic line LP44.1A-Ht/wx Reid or LP48.1B Lancaster;

d) allowing natural cross pollination to occur between the synthetic lines; and

e) harvesting seeds produced on the emasculated plants of the synthetic line.

5           9.    A synthetic hybrid corn plant designated LP57.1 and seed thereof produced by crossing a synthetic corn plant according to claim 2 and having one half the nuclear genotype of the synthetic corn plant of claim 8.

10           10.   Corn grain produced by the process of:

(a) interplanting, in pollinating proximity, seeds of synthetic hybrid corn plant LP57.1 and seeds of an elite male sterile corn hybrid;

(b) cultivating corn plants resulting from the planting;

15           (c) allowing the LP57.1 corn plants to pollinate the elite male sterile hybrid corn plants;

(d) harvesting the resulting corn grain from all plants.

20           11.   The process of claim 10 comprising the further step of emasculating the flowers of the plants of the elite male sterile hybrid by means selected from the group consisting of genetic means, cytoplasmic means and chemical means.

          12.   Synthetic corn seed designated LP44.1A-Ht/wx Reid and having an ATCC Accession No. 97888.

          13.   Synthetic corn seed designated LP48.1B Lancaster and having an ATCC Accession No. 97887.

25

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/05950**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : A01H 5/00, 4/00; C12N 5/04

US CL : 800/200, 250, DIG 56; 435/412, 424, 430, 430.1; 47/58, DIG 1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/200, 250, DIG 56; 435/412, 424, 430, 430.1; 47/58, DIG 1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN-CAPLUS, BIOSIS, DIALOG TRADEMARK DATABASES

search terms: synthetic hybrid corn, LP57.1, LP44.1a-Ht/wx Reid, LP48.1B Lancaster

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 5,675,065 A (BERGQUIST) 07 October 1997, Table 1, col. 13-15.	1-13
A	COE et al. The Genetics of Corn. In: Corn and Corn Improvement, Sprague et al. (Eds.). ASA, Inc. Madison, Wisconsin, 1988. Pages 81-137.	1-2, 4, 7-13
A	WYCH, Production of Hybrid Seed Corn. In: Corn and Corn, Improvement, Third Edition. Sprague et al. (Eds.). ASA, Inc. Madison, Wisconsin, 1988. Pages 565-607.	1-2, 4, 7-13
A	PHILLIPS et al. Cell/Tissue Culture and In Vitro Manipulation. In: Corn and Corn Improvement, Third Edition, Sprague et al. (Eds.). ASA, Inc. Madison, Wisconsin, 1988. Pages 345-387.	3, 5-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

08 MAY 1998

Date of mailing of the international search report

09 JUN 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

MELISSA KIMBALL

Telephone No. (703) 308-0196